This paper is part of the following report:

TITLE: Materials Research Society Symposium Proceedings. Volume 723.
Molecularly Imprinted Materials - Sensors and Other Devices. Symposia
Held in San Francisco, California on April 2-5, 2002

To order the complete compilation report, use: ADA412559

The component part is provided here to allow users access to individually authored sections
of proceedings, annals, symposia, etc. However, the component should be considered within
the context of the overall compilation report and not as a stand-alone technical report.

The following component part numbers comprise the compilation report:
ADP013597 thru ADP013618

MOLECULAR IMPRINTING OF POLYMERIC CORE-SHELL NANOPARTICLES

Natalia Pérez Moral and Andrew G. Mayes
School of Chemical Sciences, University of East Anglia, Norwich, NR4 7TJ, U.K.

ABSTRACT

In order to evaluate the compatibility of structured nanoparticles produced by aqueous emulsion polymerisation with the non-covalent imprinting procedure, a number of imprinted polymeric nanoparticles have been synthesised by seeded emulsion polymerisation in the presence and absence of a porogenic solvent. Propranolol was chosen as the template molecule using methacrylic acid (MAA) and ethylene glycol dimethacrylate (EDMA) as functional monomer and crosslinker respectively. The influence of the porogen and the amount of template added was studied by measuring the capacity of the polymeric particles to rebind template both in organic and aqueous buffers by radioligand binding assay. By increasing the amount of template from 0.5 to 6% (mol/mol with respect to monomers) the specific rebinding was increased from 2% to 24% in aqueous buffer and to 31% in a toluene based assay. The influence of the porosity was also established when the rebinding was performed in an organic solvent.

INTRODUCTION

Molecularly imprinted polymers (MIPs) address the need for robust, simple, fast and efficient methods to detect and separate specific molecules of interest such as drugs and pesticides. They are prepared easily and rapidly, and can be used in pre-concentration techniques, analysis, extraction, catalysis or separation. The imprinting technology consists basically of the synthesis of a highly crosslinked polymer in the presence of a template molecule. After removal of the template, the polymer is left with imprinted sites "fixed" in its structure that are complementary in shape, size and functionality to the targeted molecule, and therefore will selectively distinguish and bind such molecules when present in the medium to be processed or analysed.

Specific recognition in the imprinted polymer depends on the type of interaction established between the template and the monomer(s) prior to polymerisation, which could be covalent, non-covalent or a combination of the two. Covalent interactions are strong and more selective but there are few types sufficiently labile to be made and broken under the mild conditions required for dynamic rebinding, limiting this approach to only a few specific functional groups. The use of a combination of covalent interactions in the creation of the imprinted step and non-covalent interactions in the rebinding of the ligand has also proved to be useful in some cases, but its use is restricted by the synthetic steps required to modify the template molecule prior to polymerisation. There are numerous examples of imprinted polymers based on non-covalent interactions, a more adaptable methodology that can be used with a large number of template molecules and allows fast kinetics in the rebinding of ligands. So far, molecules have been successfully imprinted based on hydrogen bonds, electrostatic and hydrophobic interactions, albeit with some limitations encountered as a consequence of the heterogeneity of binding sites and limitations in the use of certain solvents.

An important factor in the performance of the MIP is the morphology of the polymer. Most imprinted polymers are synthesised by bulk polymerisation because of its simplicity and convenience, despite the irregularity of the particles produced, the waste of polymer and the lack

of control in the process. New methods of MIP synthesis giving control of the morphology of the polymer offer the possibility of applying MIP to new fields and conditions. For these reasons, imprinted polymers in the form of particles have been developed. The production of polymers directly in the particle format avoids the necessity of grinding them, and also allows the creation of polymers with high surface areas where diffusion of the ligands should be facilitated. Some of the new methodologies developed in recent years for imprinting of polymeric particles in organic media using non-covalent interactions are the synthesis of imprinted microsized particles in a fluorocarbonated solvent by suspension polymerisation [1] or the synthesis of nanoparticles by precipitation polymerisation [2]. In aqueous environments imprinted particles of 2-50 micron have also been described using a 2-step swelling method [3]. Emulsion polymerisation has also been used to create imprinted nanoparticles in an aqueous environment using the combination of covalent-non covalent interaction or directed imprinting at the surface of particles based on hydrophobic interactions [4,5]. To date, it has not been combined with the classical non-covalent approach. In particular, a two-stage emulsion polymerisation procedure would provide many attractive opportunities if it could be combined with non-covalent imprinting, since core-shell structures can be produced by this well-established methodology. Core-Shell methodology provides an efficient way to control and predict the final number and size, and therefore surface area, of imprinted monodisperse particles, as well as the opportunity to introduce specific properties such as fluorescence or magnetism into the cores. The binding sites should be restricted to the thin particle shells where they would have good accessibility and exchange kinetics for ligand binding.

In this paper, in order to evaluate the compatibility of core-shell nanoparticles produced by a 2-stage emulsion polymerisation system and imprinting with the versatile non-covalent imprinting procedure, we have synthesised a series of polymeric nanoparticles using a seeded emulsion polymerisation procedure. Imprinted shells have been made both in the presence and absence of a porogenic solvent. The influence of the porogen and the amount of template added were studied together with the capacity of the polymeric particles to rebind template both from organic and aqueous buffers by radioligand binding assay. Propranolol was selected as the template to be imprinted in the shell of the nanoparticles because it has been previously non-covalently imprinted by other polymerisation methods and its rebinding analysed in aqueous buffers and organic solvents.

EXPERIMENTAL SECTION

To remove the inhibitor prior to polymerisation, methacrylic acid (MAA) was distilled under vacuum, while methyl methacrylate (MMA) and ethylene glycol dimethacrylate (EDMA) were washed with 1M aqueous sodium hydroxide, dried over $MgSO_4$ and stored with molecular sieves at 4 °C until required. Sodium dodecyl sulfate (SDS), ammonium peroxodisulfate (APS) and other solvents and reagents were used as received. ^{3}H-propranol (specific activity 29Ci/mmol, 1μCi/ μL in ethanol) was obtained from Sigma. Scintillation counting was performed in a Wallac 1409 DSA β-radiation counter. Scintillation cocktail Ecoscint A (National Diagnostics) was used for aqueous samples. Organic samples were counted in toluene containing 2,5 diphenyloxazol (PPO - 3g/l) and 1,4-bis (5phenyloxazol- 2-yl)-benzene (POPOP - 0.2g/l), both obtained from Aldrich. Particles were characterised by T.E.M. using a Jeol JEM 200E X Transmission Electron Microscope. Samples were prepared by drying a drop of a solution of particles in water with SDS over a grid coated by a carbon film.

Synthesis of particles

The seeds were prepared using a standard batch emulsion polymerisation in a 1 litre three-necked jacketed reactor connected to a water bath to control the temperature. The system was equipped with a condenser, a mechanical stirrer and a gas inlet to maintain an inert argon atmosphere. A solution of $NaHCO_3$ (0.95 gr.; 11.3 mmol) in distilled water (520 gr.) with SDS (0.913gr.; 3.16 mmol) was added into the reactor and purged with argon to remove oxygen under gentle stirring, while increasing the temperature to 90 °C. Once the temperature was reached, the monomer mixture was added and the stirring speed increased to 600 rpm. After 1 minute, the initiator APS was added dissolved in 1 ml of water to initiate the polymerisation. The temperature was maintained at 90 °C for 24 hours to ensure total decomposition of the initiator. The final latex was filtered through a fine nylon mesh and its solid content calculated before being used in the next step.

Core-shell particles were synthesised using a 500-ml reactor similar to that described above. In a typical reaction, firstly, a solution containing water (88.5 gr.) and SDS (0.5gr.; 1.73 mmol) was added into the reactor and purged with argon under gentle stirring while the temperature was raised to 70 °C. A solution containing a mixture of monomers (EDMA 10.1gr; 50.9 mmol; MAA 1.1 gr.; 12.7 mmol), porogen (10.6 ml toluene), template and seed (51.4 gr.) that had been previously mixed for 20 minutes, was also charged into the reactor followed by an aqueous solution of 0.112 gr (0.84 mmol) of APS. The stirring speed was increased to 200 rpm and the reaction allowed to proceed for 6 hours before cooling to room temperature.

The resultant polymer particles were ultrafiltered to remove any surfactant adsorbed on the particles, and then the template was removed by washes with ammonium acetate 1M dissolved in a mixture of ethanol (40) / acetic acid (25) / water (35), followed by washes in acetic acid/ ethanol 1:3 and methanol [7].

Radioligand Binding Assay

0.25mg of particles were mixed with 1ml of a solution of ^{3}H-propranolol (0.07 µl H^3-propranolol ml^{-1}) in the solvent mixture (toluene with 0.5% acetic acid or aqueous solution of sodium citrate 25 mM with 0.5% acetic acid and 2% ethanol) and incubated overnight at room temperature. The solution was then centrifuged at 12000 rpm for 5 minutes, and 0.5 ml of the supernatant mixed with 3 ml of scintillation liquid. The radioactivity was measured by liquid scintillation counting.

RESULTS AND DISCUSSION

Synthesis of core-shell particles

Polymer particles made in a 2-stage emulsion polymerisation system can produce structured particles with a core-shell arrangement if conditions are chosen appropriately. A latex obtained by a standard batch emulsion polymerisation was used as a seed to polymerise a crosslinked shell polymer over it in a second stage. By assigning the amount of seed polymer, and as long as secondary nucleation is restricted, the final number, size and size distribution of particles can be controlled.

Core-shell particles are not always produced, however, when polymer particles are synthesised in two successive stages. Various alternative outcomes, such as nucleation of new

particles or different distributions of the components ("raspberry-like", "acorn like") can also occur [6]. The final particle structure is determined by the influence of thermodynamic and kinetic stability forces between the components of the polymer. In order to find a good system to promote the creation of core-shell particles, two different seeds were synthesised and their ability to generate imprinted shells were assessed (see table I).

Table I. Synthesis of core particles. Polymerisation conditions: 90 °C for 24 hours, initiatior= ammonium persulfate; S.C.= solid content, Dp= particle diameter.

	Monomer(s) (mol %)	SDS/M (mol %)	S.C. (%)	Dp (nm)	Nature of latex
1	MMA: EDMA 95/5	0.84	7.8%	53	monodisperse
2	MMA 100	0.84	7%	55	monodisperse

Both of the resultant polymers were assessed by TEM, which showed that the latices were monodisperse in size, with diameters in the order of 55nm when the core was made 100% of MMA and 53 nm when the core was 5% crosslinked with EDMA.

After being characterised, each of these latices was used as a seed in a second stage of the polymerisation to create a highly crosslinked shell around it. Attempts to create a core-shell structure over the MMA core under the chosen conditions were not successful, resulting in high viscosity and low conversion of monomers to polymers. On the other hand, the core-shell arrangement was kinetically favoured when a small level of crosslinker was incorporated into the core, yielding a high conversion latex. Consequently, core 1 was selected as the seed for the imprinting of shells in the second stage.

The influence of porogen (toluene) and template was studied by preparing different core-shell particles varying the amount of propranolol added in the second stage of polymerisation from 0.5% to 6%, with and without porogen (Table II). Control particles were also synthesised in the absence of propranolol.

Table II. Synthesis of core-shell particles using core 1 as seed.
(prop = propranolol, M = MAA+EDMA, porogen = toluene, porogen/monomer ratio = 1/1 (vol/vol), Dp = particle diameter).

Composition of polymer		Dp (nm)	
% prop/M (mol)	MAA/prop (mol)	with porogen	without porogen
----	----	70	60
6	3.4 / 1	72	
3.9	5 / 1	66	60
2	10 / 1	70	52
1	20 / 1	55	59
0.5	40 / 1	37	60

In general, the particle sizes (measured from the TEM images) were as expected based on the seed size and monomer charge during shell synthesis. In a few cases, however, notably in porous polymer imprinted with 0.5% of propranolol and to a certain extent in non-porous

polymer imprinted with 2% propranolol (visible in figure 1e), secondary nucleation occurred giving a population of smaller particles which reduced the average Dp below that expected.

Particles synthesised in the presence of a porogenic solvent are expected to have a porous shell with higher surface area than the particles synthesised in its absence. In general terms, porous particles present a slightly wider polydispersity in size. Some electron micrographs of the particles are shown in Figure 1.
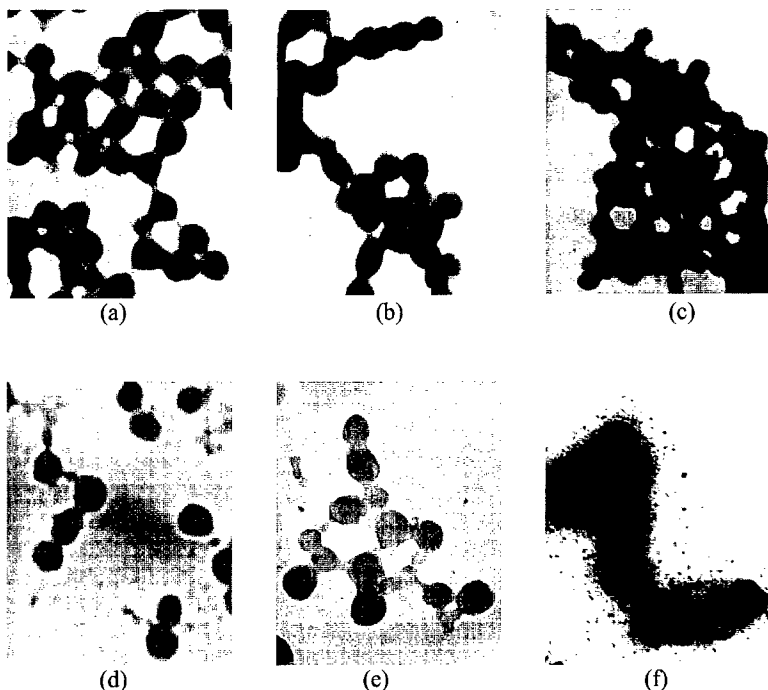

Figure 1. TEM photographs of (a) seed 1; (b) non-imprinted porous core-shell; (c) 3.9% imprinted porous core-shell; (d) non-imprinted non-porous core-shell; (e) 2% imprinted non-porous core-shell and (f) 3.9% imprinted non-porous core-shell.

The capacity of the imprinted polymers to rebind propranolol from organic and aqueous solutions was analysed by radioligand binding assay. Results are presented in table III. As expected, it was found that the rebinding of propranolol to MIP increases with the amount of template incorporated into the shell. When the imprinted sites are made more accessible to the ligands by incorporating porosity into the shell, this effect is more significant. The specific rebinding of propranolol (defined as the amount of ligand rebound to the imprinted polymer after subtraction of the amount rebound non-specifically to non-imprinted particles) in toluene containing 0.5% acetic acid was higher than when the analysis was performed in an aqueous buffer, but in both cases the imprinting effect was significant.

The particles that contain a porous shell showed a higher uptake of propranolol than the "compact" non-porous imprinted shells. Presumably, the latter particles have the imprinted sites situated at or very near the surface, but they still bind significant levels of propranolol compared to control particles. More detailed studies are underway to determine the effect of shell porosity on site accessibility, exchange kinetics and the balance of forces involved in the binding interactions.

Table III. Specific rebinding of propranolol ((rebinding to imprinted particles) – (rebinding to non-imprinted particles)) to 0.25 mg.of polymer, buffer = Na citrate 25mM + 0.5% acetic acid + 2% ethanol pH = 4.2, toluene* = toluene + 0.5% acetic acid ; values are means of 4 replicates.

Polymer	% Specific rebinding in buffer		% Specific rebinding in toluene*	
% prop/M	particles with porogen	particles without porogen	particles with porogen	particles without porogen
(----)	(16)	(11)	C(2)	(16)
6	24		31	
3.9	19	14	27	8
2	18	10	15	8
1	15	6	9	9
0.5	2	3	2	3

CONCLUSIONS

It has been demonstrated that non-covalent imprinting of templates is possible in the shells of core-shell nanoparticles, despite the fact that they are synthesised in the presence of an aqueous continuous phase and the organic imprinting phase must be saturated with water. The binding capacity is somewhat lower than that usually measured for bulk imprinting of propranolol, but this can no doubt be increased by careful optimisation of the many system variables such as template concentration and continuous phase pH. Including a porogenic solvent during shell synthesis increased the binding capacity of the particles, but significant rebinding was also shown by the non-porous shells. Further studies are under way to characterise the effect of the porogen in greater detail, and to measure its effect on binding site accessibility, selectivity and exchange kinetics.

REFERENCES

1. A. G. Mayes and K. Mosbach, *Anal. Chem.* **68**, 3769-3774 (1996).
2. L. Ye, P. A. G. Cormack and K. Mosback, *Anal. Commun.* **36**, 35-38 (1999).
3. K. Hosoya, K. Yoshizako, N. Tanaka, K. Kimata, T. Araki and J. Haginaka, *Chemistry. Letters*, 1437-1438 (1994).
4. N. Perez, M. J. Whitcombe and E. N. Vulfson, *J. Appl. Polym. Sci.* **77**, 1851-1859 (2000).
5. N. Perez, M. J. Whitcombe and E. N. Vulfson, *Macromolecules* **34**, 830-836 (2001).
6. S. Lee and A. Rudin in *Polymer Latexes*, edited by E. S. Daniels, E. D. Sudol and M. S. El-Aaser, (ACS Symposium series 492, 1992) pp 234-254.
7. L. I Andersson, *Anal. Chem.* **68**, 111-117 (1996).